abcam

ab32351

Anti-Caspase-3 antibody [E87]

Anti-Caspase-3 antibody [E87] (ab32351) is a rabbit monoclonal antibody detecting Caspase-3 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 380 publications
- Trusted since 2006

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E87
Purification technique	Affinity purification Protein A
Specificity	This antibody is specific for the pro form and the p17 cleaved form of human Caspase-3.
Concentration	0.902 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/25 - 1/100
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

IP

Tested

Species	Human
Dilution info	1/10 - 1/50
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/5000
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1 µg/mL
Notes	**For unpurified, use 1/25 dilution.**

Not recommended

Species	Mouse
Dilution info	-
Notes	**For unpurified, use 1/25 dilution.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/180 - 1/1000
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Mouse
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information CASP3

Function	Thiol protease that acts as a major effector caspase involved in the execution phase of apoptosis (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:35338844, PubMed:35446120, PubMed:7596430). Following cleavage and activation by initiator caspases (CASP8, CASP9 and/or CASP10), mediates execution of apoptosis by catalyzing cleavage of many proteins (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:7596430). At the onset of apoptosis, it proteolytically cleaves poly(ADP-ribose) polymerase PARP1 at a '216-Asp-|-Gly-217' bond (PubMed:10497198, PubMed:16374543, PubMed:7596430, PubMed:7774019). Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain (By similarity). Cleaves and activates caspase-6, -7 and -9 (CASP6, CASP7 and CASP9, respectively) (PubMed:7596430). Cleaves and inactivates interleukin-18 (IL18) (PubMed:37993714, PubMed:9334240). Involved in the cleavage of huntingtin (PubMed:8696339). Triggers cell adhesion in sympathetic neurons through RET cleavage (PubMed:21357690). Cleaves DSG2 in response to apoptosis resulting in a loss of full length DSG2 at desmosome cell junctions and subsequent loss of cell-cell adhesion (PubMed:17559062). Also cleaves JUP in response to apoptosis (PubMed:17559062). Cleaves and inhibits serine/threonine-protein kinase AKT1 in response to oxidative stress (PubMed:23152800). Acts as an inhibitor of type I interferon production during virus-induced apoptosis by mediating cleavage of antiviral proteins CGAS, IRF3 and MAVS, thereby preventing cytokine overproduction (PubMed:30878284). Also involved in pyroptosis by mediating cleavage and activation of gasdermin-E (GSDME) (PubMed:35338844, PubMed:35446120). Cleaves XRCC4 and phospholipid scramblase proteins XKR4, XKR8 and XKR9, leading to promote phosphatidylserine exposure on apoptotic cell surface (PubMed:23845944, PubMed:33725486). Cleaves BIRC6 following inhibition of BIRC6-caspase binding by DIABLO/SMAC (PubMed:36758104, PubMed:36758106).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-Caspase-3 antibody [E87] (ab32351) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, IP, WB in human samples.

Anti-Caspase-3 antibody [E87] (ab32351) has been cited over 386 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-Caspase-3 antibody [E87] (ab32351) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-Caspase-3 antibody [E87] (ab32351) has been confirmed by testing in knockout samples.

Anti-Caspase-3 antibody [E87] (ab32351) has 11 independent reviews from customers.

Anti-Caspase-3 antibody [E87] (ab32351) specifically detects Caspase-3 (UniProt ID: P42574; Molecular weight: 17kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone E87 - ab197202.

Antibody clone E87 is also available pre-conjugated to a variety of labels for your convenience - Biotin, PE, APC, HRP, Alkaline Phosphatase, Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 594 (ab195905, ab305665, ab305666, ab305667, ab308706, ab309678, ab310040, ab310425).

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

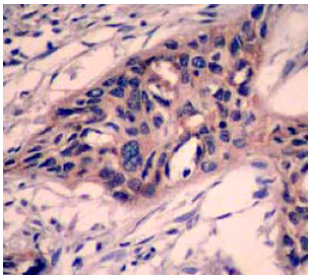
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

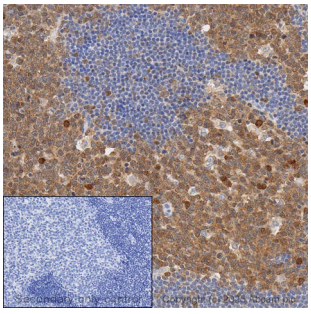
12 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Caspase-3 antibody

Unpurified ab32351, at a 1/25 dilution, staining Capase-3 in paraffin embedded human cervical carcinoma tissue by Immunohistochemistry.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



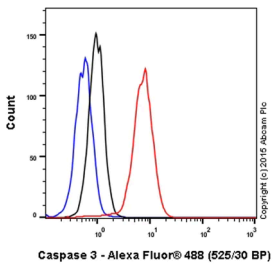
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human Tonsil tissue using rabbit Anti-Caspase-3 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling caspase-3 with ab32351 at a concentration of 0.1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab32351 anti-Caspase-3 antibody [E87] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

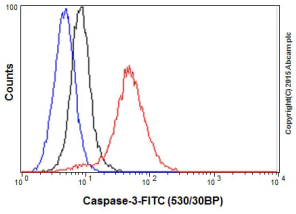
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-3 antibody

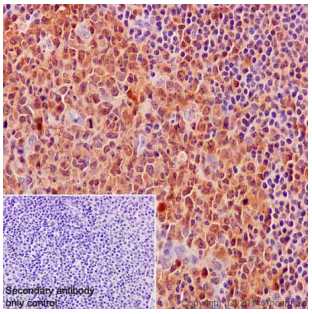
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified ab32351 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32351, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr®488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730, 0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-3 antibody

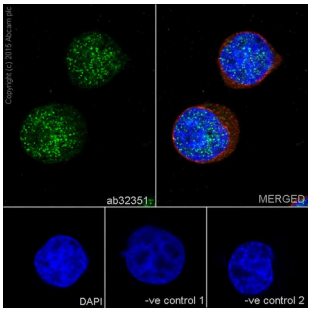
Overlay histogram showing Ramos (human Burkitt's lymphoma cell line) cells fixed in 4% PFA and stained with purified ab32351 at a dilution of 1 in 180 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Caspase-3 antibody

Immunohistochemical staining of paraffin embedded human tonsil with purified ab32351 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

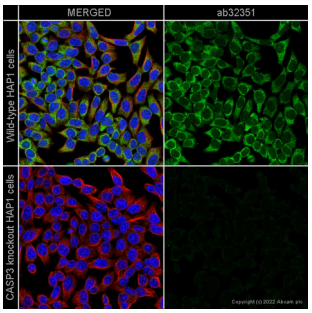


Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Caspase-3 antibody

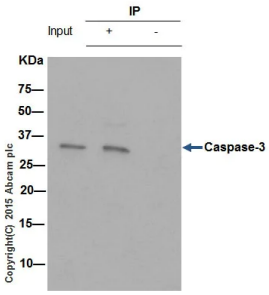
Immunofluorescence staining of Jurkat (human T cell leukemia cell line from peripheral blood) cells with purified ab32351 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32351 was used at a dilution of

1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] (ab32351)

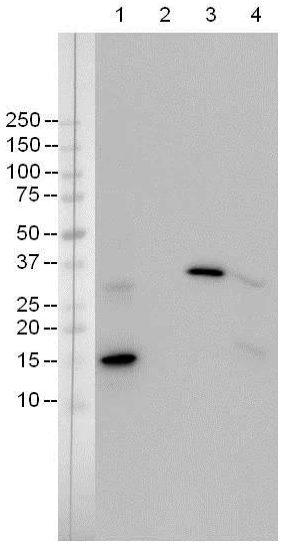
ab32351 staining Caspase-3 in wild-type Hap1 cells (top panel) and CASP3 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32351 at 1µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunoprecipitation - Anti-Caspase-3 antibody [E87] (ab32351)

ab32351 (purified) at 1/50 immunoprecipitating Cullin 1 in 10 µg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate (Lanes 1 and 2, observed at 35 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST
All lanes:
Immunoprecipitation - Anti-Caspase-3 antibody [E87] (ab32351)

Predicted band size: 31 kDa

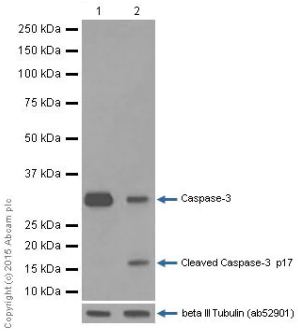


Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Western blot staining using rabbit Anti-Caspase-3 antibody

Carried out with unpurified antibody. Lane 1 = Caspase 3 protein (Active) (ab52314) 20 ng. Lane 2 = Caspase 9 protein (Active) (ab52203) 20 ng. Lane 3 = Extract of HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with vehicle (ab136806) 20 ug. Lane 4 = Extract of HeLa cells treated with staurosporine (ab136806) 20 ug. SDS PAGE performed under reducing conditions (100 mM DTT Sample heated at 50°C). Primary : Lanes 1-4: Anti Caspase 3 antibody (ab32351) at 1:1000 dilution. Secondary : Lanes 1-4: Goat anti rabbit IgG(H&L)-HRP at 1:10000. Development: ECL for 10 min exposure. Blocking: in 5% Milk + PBS overnight at 4 C. Primary antibody: in 5% Milk + PBS for 2 hours at RT. Secondary antibody: in 5% Milk + PBS for 2 hours at RT. Predicted band size : 32 kDa and 17 kDa. Observed band size : 32 kDa and 17 kDa.
All lanes:
Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

Predicted band size: 31 kDa



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

Caspase-3 Western blot staining using rabbit Anti-Caspase-3 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution

Lane 1:
untreated Jurkat (human T cell leukemia cell line from peripheral blood)cell lysate at 10 µg

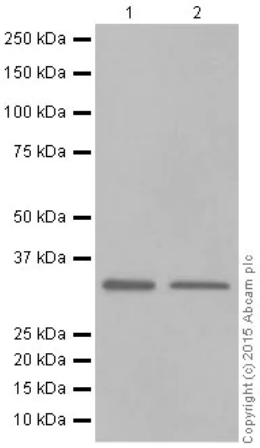
Lane 2:
Jurkat treated with staurosporine at 10 µg

Secondary

All lanes:
HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 31 kDa

Observed band size: 17 kDa, 35 kDa



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution

Lane 1:
Ramos (human Burkitt's lymphoma cell line) cell lysate at 20 µg

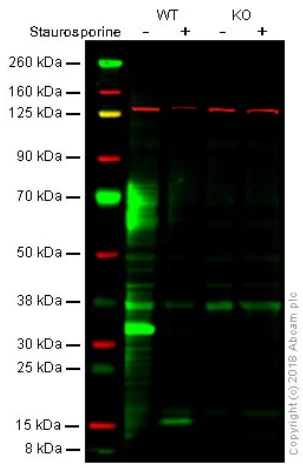
Lane 2:
HEK-293 (human epithelial cell line from embryonic kidney) cell lysate at 20 µg

Secondary

All lanes:
HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 31 kDa

Observed band size: 35 kDa



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

Lanes 1 - 4: Merged signal (red and green). Green - ab32351 observed at 31 kDa. Red - loading control, ab130007, observed at 130 kDa.
ab32351 was shown to recognize Caspase 3 in wild-type HAP1 cells as signal was lost at the expected MW in HAP1 Staurosporine Treated (CASP3) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HAP1 Staurosporine Treated (CASP3) knockout samples were subjected to SDS-PAGE. ab32351 and ab130007 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution

Lane 1:
DMSO control wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
Staurosporine treated wild-type HAP1 whole cell lysate at 20 µg

Lane 3:
DMSO control CASP3 knockout HAP1 whole cell lysate at 20 µg

Lane 4:
Staurosporine treated CASP3 knockout HAP1 whole cell lysate at 20 µg

Predicted band size: 31 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.